

SEQUENCE LISTING

<110> Commonwealth Scientific and Industrial Research Organization

<120> Efficient gene silencing in plants using short dsRNA sequences

<130> BCS-03-2001

<150> US 60/447,711

<151> 2003-02-19

<160> 33

<170> PatentIn version 3.1

<210> 1

<211> 341

<212> DNA

<213> Artificial Sequence

<220>

<223> sequence of the promoter of the 7SL-2 gene of Arabidopsis thaliana
a var. Landsberg erecta

<220>

<221> misc_feature

<222> (1)..(6)

<223> XhoI restriction site

<220>

<221> misc_feature

<222> (7)..(322)

<223> PolIII promoter region

<220>

<221> misc_feature

<222> (336)..(341)

<223> XhoI restriction site

<220>

<221> misc_feature

<222> (329)..(335)

<223> poly T nucleotide stretch

<220>

<221> misc_feature

<222> (323)..(328)

<223> SalI restriction site

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ctcgagatgt	tgttggtacc	agaaagtaaa	taaatgttca	atctctgatg	ttctcaagta	60
agtgaagttt	attgggaata	atattaactc	atgttcttct	gcatttgatt	cctttgccgc	120
tctcttcttc	tatcttaa	at ctgtgtatac	tatttcacta	ttgggctttt	tattagtcta	180
taatgggact	caaaataagg	c ttgtggcca	catcaaaaag	ataagtcaca	aatcaaaact	240
aaattcagag	tcttttctcc	cacatcggtc	actgtactca	ttttgtgttt	gtttatatat	300
tacacgaacc	gatctttgtt	acgtcgactt	tttttctcga	g		341

<210> 2

<211> 429

<212> DNA

<213> Artificial Sequence

<220>

<223> sequence of the promoter of the 7SL-2 gene of Arabidopsis thaliana var. Landsberg erecta including 86 bases downstream of the transcription initiation site.

<220>

<221> misc_feature
 <222> (1)..(6)
 <223> XhoI restriction site

<220>

<221> misc_feature
 <222> (424)..(429)
 <223> XhoI restriction site

<220>

<221> misc_feature
 <222> (415)..(423)
 <223> poly T stretch

<220>

<221> misc_feature
 <222> (409)..(414)
 <223> SalI restriction site

<220>

<221> misc_feature
 <222> (7)..(408)
 <223> PolIII promoter region.

<400> 2
 ctcgagatgt tgttggtacc agaaagtaaa taaatgttca atctctgatg ttctcaagta 60
 agtgagtttt attgggaata atattaactt atgttcttct tgcatttgat ttctttgccg 120
 ctctcttctt ctatcttaaa tctgtgtata ctatttcact attgggcttt ttattagtct 180
 ataatgggac tcaaaataag gctttggccc acatcaaaaa gataagtcac aaatcaaaac 240
 taaattcaga gtcttttctc ccacatcggt cactgtactc ttttgtgttt gtttatatat 300
 tacacgaacc gatctttggt acgtcgagct aagtaacatg agcttgtaac ccatgtgggg 360
 acattaagat ggtggaacac tggctcgggt ccacgggccg gttctgttgt cgactttttt 420
 tttctcgag 429

<210> 3

<211> 334
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<220>

<223> sequence of the promoter of the U3 snRNA of *Arabidopsis thaliana* var. *Landsberg erecta*

<220>

<221> misc_feature
<222> (1)..(6)
<223> EcoRI restriction site

<220>

<221> misc_feature
<222> (314)..(319)
<223> PvuI restriction site

<220>

<221> misc_feature
<222> (320)..(328)
<223> poly T stretch

<220>

<221> misc_feature
<222> (329)..(334)
<223> EcoRI restriction site

<220>

<221> misc_feature
<222> (7)..(313)
<223> Pol III promoter region

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<400> 3
gaattcttat gcagcctgtg atggataact gaatcaaaca aatggcgtct gggtttaaga      60
agatctgttt tggctatggt ggacgaaaca agtgaacttt taggatcaac ttcagtttat      120
atatggagct tatatcgagc aataagataa gtgggctttt tatgtaattt aatgggctat      180
cgtccataga ttcactaata cccatgccca gtacccatgt atgcgtttca tataagctcc      240
taatttctcc cacatcgctc aaatctaaac aaatcttggt gtatatataa cactgagggga      300
gcaacattgg tcacgatcgt ttttttttga attc                                     334

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<210> 4

<211> 467

<212> DNA

<213> Artificial Sequence

<220>

<223> sequence of the promoter of the U3 snRNA gene of *Arabidopsis thaliana* var. *Landsberg erecta* including 136 bases downstream of the transcription initiation site.

<220>

<221> misc_feature

<222> (1)..(6)

<223> EcoRI restriction site

<220>

<221> misc_feature

<222> (7)..(446)

<223> Pol III promoter region

<220>

<221> misc_feature

<222> (447)..(452)

<223> XhoI restriction site

<220>

<221> misc_feature

<222> (453)..(461)

<223> poly T stretch

<220>

<221> misc_feature

<222> (462)..(467)

<223> EcoRI restriction site

<400> 4

gaattcttat	gcagcctgtg	atggataact	gaatcaaaca	aatggcgtct	gggtttaaga	60
agatctgttt	tggtatgtt	ggacgaaaca	agtgaacttt	taggatcaac	ttcagtttat	120
atatggagct	tatatcgagc	aataagataa	gtgggctttt	tatgtaattt	aatgggctat	180
cgtccataga	ttcactaata	cccatgccca	gtacccatgt	atgcgtttca	tataagctcc	240
taattttctcc	cacatcgctc	aaatctaaac	aaatcttggt	gtatatataa	cactgagggga	300
gcaacattgg	tcacgacctt	acttgaacag	gatctgttct	ataggctcgt	acctctgttt	360
ccttgatttc	tcaagagaca	ggcccttaac	cctggttgat	gaaccatgac	cgtgcggcta	420
gagcgtgatt	gacggctacg	atcgtcctcg	agtttttttt	tgaattc		467

<210> 5

<211> 456

<212> DNA

<213> Artificial Sequence

<220>

<223> sequence of the promoter of the U6 snRNA gene of Arabidopsis thaliana var. Landsberg erecta including 3 bases downstream of the transcription initiation site

<220>

<221> misc_feature

<222> (1)..(6)

<223> XhoI restriction site

<220>

<221> misc_feature

<222> (7)..(436)

<223> Pol III promoter region

<223> SalI restriction site

<223> poly T stretch

<223> Sac I restriction site

<213> Artificial Sequence

<220>

<221> misc_feature
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 <223> XhoI restriction site

<220>

<221> misc_feature
 <222> (7)..(468)
 <223> Pol III promoter region

<220>

<221> misc_feature
 <222> (469)..(474)
 <223> PvuI restriction site

<220>

<221> misc_feature
 <222> (475)..(482)
 <223> Poly T stretch

<220>

<221> misc_feature
 <222> (483)..(488)
 <223> XhoI restriction site

<400> 6
 ctcgagcttc gttgaacaac ggaaactcga cttgccttcc gcacaataca tcatttcttc 60
 ttagcttttt ttcttcttct tcgttcatac agtttttttt tgtttatcag cttacatttt 120
 cttgaaccgt agctttcggt ttcttctttt taactttcca ttcgaggttt ttgtatcttg 180
 tttcatagtt tgtcccagga ttagaatgat taggcacga accttcaaga atttgattga 240
 ataaaacatc ttcattctta agatatgaag ataactctca aaaggcccct gggaatctga 300
 aagaagagaa gcaggcccat ttatatggga aagaacaata gtatttctta tataggccca 360
 tttaagtga aaacaatctt caaaagtccc acatcgctta gataagaaaa cgaagctgag 420
 tttatataca gctagagtcg aagtagtgat tgtcccttcg gggacatccg atcgtttttt 480
 ttctcgag 488

<210> 7

<211> 405

<212> DNA

<213> Artificial Sequence

<220>

<223> sequence of the promoter of the U3 snRNA of rice (*Oryza sativa* Indica IR36)

<220>

<221> misc_feature

<222> (1)..(6)

<223> EcoRI restriction site

<220>

<221> misc_feature

<222> (7)..(384)

<223> Pol III promoter region

<220>

<221> misc_feature

<222> (385)..(390)

<223> PvuI restriction site

<220>

<221> misc_feature

<222> (391)..(399)

<223> poly T stretch

<220>

<221> misc_feature

<222> (400)..(405)

<223> EcoRI restriction site

<400> 7

gaattcaagg	gatcttttaa	catacgaaca	gatcacttaa	agttcttctg	aagcaactta	60
aagttatcag	gcatgcatgg	atcttgagg	aatcagatgt	gcagtcaggg	accatagcac	120
aggacaggcg	tcttctactg	gtgctaccag	caaatgctgg	aagccgggaa	cactgggtac	180
gttggaaacc	acgtgatgtg	gagtaagata	aactgtagga	gaaaagcatt	tcgtagtggg	240
ccatgaagcc	tttcaggaca	tgtattgcag	tatgggccgg	cccattacgc	aattggacga	300
caacaaagac	tagtattagt	accacctcgg	ctatccacat	agatcaaagc	tggtttaaaa	360
gagttgtgca	gatgatccgt	ggcacgatcg	tttttttttg	aattc		405

<210> 8

<211> 442

<212> DNA

<213> Artificial Sequence

<220>

<223> sequence of the promoter of the U3 snRNA of tomato (a garden variety with small gourd-shaped yellow fruit)

<220>

<221> misc_feature

<222> (1)..(6)

<223> EcoRI restriction site

<220>

<221> misc_feature

<222> (7)..(421)

<223> Pol III promoter region

<220>

<221> misc_feature

<222> (422)..(427)

<223> PvuI restriction site

<220>

<221> misc_feature

<222> (428)..(436)

<223> Poly T stretch

<220>

<221> misc_feature

<222> (437)..(442)

<223> EcoRI restriction site

<400> 8

gaattctgag	agcatttgt	ggcgttcctc	tgaattactt	actgtcactt	tgattggagc	60
cattattttc	agactctact	gaagattgaa	ttgaatgaga	aactatgaaa	ctttacaagt	120
gaattattat	ggagttcatg	gcaactgcta	tggagttttt	cctactggga	attggaacgg	180
tttctacgaa	attaactgtc	cacacgttaa	aaatataaat	taatgcgtaa	ttgttatttt	240
ttctataaca	aataaaaaac	tgaaatacga	cataaatttt	attactttta	ttgcacttta	300
gccttagaga	tattgcgttg	tagtcggcgt	aggtgtgtca	ggggccaata	tattgttccc	360
acatcggcag	tcagcacat	aaactctagc	gttataagaa	tctatccact	atcaacggtc	420
acgatcgttt	ttttttgaat	tc				442

<210> 9

<211> 295

<212> DNA

<213> Artificial Sequence

<220>

<223> sequence of the dsRNA encoding region of 94bp for silencing expression of the GUS gene (GUShp94)

<220>

<221> misc_feature

<222> (1)..(6)

<223> SalI restriction site

<220>

<221> misc_feature

<222> (6)..(11)

<223> PvuI restriction site

<220>

<221> misc_feature

<222> (12)..(100)

<223> GUS sequence (sense)

<220>

<221> misc_feature

<222> (101)..(195)

<223> spacer sequence

<220>

<221> misc_feature

<222> (190)..(195)

<223> BamHI restriction site

<220>

<221> misc_feature

<222> (196)..(284)

<223> GUS sequence (antisense)

<220>

<221> misc_feature

<222> (285)..(290)

<223> PvuI restriction site

<220>

<221> misc_feature

<222> (290)..(295)

<223> SalI restriction site

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<400> 9
gtcgacgacgc gcagcgtaat gctctacacc acgccgaaca cctgggtgga cgatatcacc 60
gtgggtgacgc atgtcgcgca agactgtaac cacgcgtctg ttgactggca ggtgggtggcc 120
aatgggtgatg tcagcggtga actgcgatgat gcggatcaac aggtgggtgc aactggacaa 180
ggcactagcg ggatccagac gcgtgggttac agtcttgccg gacatgcgtc accacggtga 240
tategtccac ccaggtgttc ggcgtgggtg agagcattac gctgcgatcg tcgac 295

```

<210> 10

<211> 93

<212> DNA

<213> Artificial Sequence

<220>

<223> sequence of the dsRNA encoding region of 41 bp for silencing expression of the GUS gene (GUShp41)

<220>

<221> misc_feature

<222> (1)..(6)

<223> SalI restriction site

<220>

<221> misc_feature

<222> (7)..(42)

<223> GUS sequence (sense)

<220>

<221> misc_feature

<222> (43)..(51)

<223> spacer sequence

<220>

<221> misc_feature

<222> (52)..(87)

<223> GUS sequence (antisense)

<220>

<221> misc_feature

<222> (88)..(93)

<223> Sal I restriction site

<400> 10

gtcgactggg cagatgaaca tggcatcgtg gtgattgatg aatgcgagaa cttcatcaat 60
caccacgatg ccatgttcat ctgcccagtc gac 93

<210> 11

<211> 50

<212> DNA

<213> Artificial Sequence

<220>

<223> sequence of the dsRNA encoding region of 21 bp for silencing expression of the GUS gene (GUShp21)

<220>

<221> misc_feature

<222> (1)..(6)

<223> SalI restriction site

<220>

<221> misc_feature

<222> (7)..(22)

<223> GUS sequence (sense)

<220>

<221> misc_feature

<222> (23)..(28)

<223> spacer sequence

<220>

<221> misc_feature

<222> (29)..(44)

<223> GUS sequence (antisense)

<220>

<221> misc_feature

<222> (45)..(50)

<223> Sal I restriction site

<400> 11

gtcgactggg cagatgaaca tgtacgatca tgttcactctg cccagtcgac

50

<210> 12

<211> 94

<212> DNA

<213> Artificial Sequence

<220>

<223> sequence of the dsRNA encoding region of 42 bp for silencing expression of the PHYB gene, derived from the 5' end of PHYB (PHYB5hp 42)-upper strand

<400> 12

tgcacggagt cgggggtagt ggcggtggcc gtggcggtgg ccgtggagga ggccacggcc
accgccacgg ccaccgccac taccgccgac tccg

60

94

<210> 13

<211> 94

<212> DNA

<213> Artificial Sequence

<220>

<223> sequence of the dsRNA encoding region of 42 bp for silencing expr

ession of the PHYB gene, derived from the 5' end of PHYB (PHYB5hp
42)-lower strand

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<400> 13
tcgacggagt cgggggtagt ggcggtggcc gtggcggtgg ccgtggcctc ctccacggcc 60
accgccacgg ccaccgccac taccgccgac tccg 94
```

<210> 14

<211> 52

<212> DNA

<213> Artificial Sequence

<220>

<223> sequence of the dsRNA encoding region of 21 bp for silencing expr
ession of the PHYB gene, derived from the 5' end of PHYB (PHYB5hp
21)-upper strand

```
<400> 14
tcgacggagt cgggggtagt ggcggaggag gccgccacta ccccgactc cg 52
```

<210> 15

<211> 52

<212> DNA

<213> Artificial Sequence

<220>

<223> sequence of the dsRNA encoding region of 21 bp for silencing expr
ession of the PHYB gene, derived from the 5' end of PHYB (PHYB5hp
21)-lower strand

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<400> 15
tcgacggagt cgggggtagt ggcggcctcc tccgccacta ccccgactc cg 52
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<210> 16

<211> 94

<212> DNA

<213> Artificial Sequence

<220>

<223> sequence of the dsRNA encoding region of 42 bp for silencing expr

ession of the PHYB gene, derived from the center of PHYB (PHYBChp 42)-upper strand

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<400> 16
tcgatggatg gtgtggttca gccatgtagg gatatggcgg gggaacagga gggttccccc 60
gccatatccc tacatggctg aaccacacca tcca 94
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<210> 17

<211> 94

<212> DNA

<213> Artificial Sequence

<220>

<223> sequence of the dsRNA encoding region of 42 bp for silencing expression of the PHYB gene, derived from the center of PHYB (PHYBChp 42)-lower strand

```
<400> 17
tcgatggatg gtgtggttca gccatgtagg gatatggcgg gggaaccctc ctgttccccc 60
gccatatccc tacatggctg aaccacacca tcca 94
```

<210> 18

<211> 52

<212> DNA

<213> Artificial Sequence

<220>

<223> sequence of the dsRNA encoding region of 21 bp for silencing expression of the PHYB gene, derived from the center of PHYB (PHYBChp 21)-upper strand

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<400> 18
tcgatggatg gtgtggttca gccataggag gatggctgaa ccacacctcc aa 52
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<210> 19

<211> 52

<212> DNA

<213> Artificial Sequence

<220>

<223> sequence of the dsRNA encoding region of 21 bp for silencing expression of the PHYB gene, derived from the center of PHYB (PHYBChp 21)-lower strand

<400> 19
tcgatggatg gtgtggttca gccatcctcc tatgggtgaa ccacaccatc ca 52

<210> 20

<211> 94

<212> DNA

<213> Artificial Sequence

<220>

<223> sequence of the dsRNA encoding region of 42 bp for silencing expression of the PHYB gene, derived from the 3' end of PHYB (PHYB3hp 42)-upper strand

<400> 20
tcgacattgt caactgctag tggaagtggg gacatgatgc tgatgaagga ggatcatcagc 60
atcatgtcac cacttccact agcagttgac aatg 94

<210> 21

<211> 94

<212> DNA

<213> Artificial Sequence

<220>

<223> sequence of the dsRNA encoding region of 42 bp for silencing expression of the PHYB gene, derived from the 3' end of PHYB (PHYB3hp 42)-lower strand

<400> 21
tcgacattgt caactgctag tggaagtggg gacatgatgc tgatgacctc ctatcatcagc 60
atcatgtcac cacttccact agcagttgac aatg 94

<210> 22

<211> 52

<212> DNA

<213> Artificial Sequence

<220>

<223> sequence of the dsRNA encoding region of 21 bp for silencing expression of the PHYB gene, derived from the 3' end of PHYB (PHYB3hp 21)-upper strand

<400> 22

tcgacattgt caactgctag tggaaaggag gttccactag cagttgacaa tg 52

<210> 23

<211> 52

<212> DNA

<213> Artificial Sequence

<220>

<223> sequence of the dsRNA encoding region of 21 bp for silencing expression of the PHYB gene, derived from the 3' end of PHYB (PHYB3hp 21)-lower strand

<400> 23

tcgacattgt caactgctag tggaacctcc tttccactag cagttgacaa tg 52

<210> 24

<211> 94

<212> DNA

<213> Artificial Sequence

<220>

<223> sequence of the dsRNA encoding region of 42 bp for silencing expression of the PDS gene (PDS42)-upper strand

<400> 24

tcgacttaac ttgtaaggaa tattacgata ctaaccggtc aatgctagga ggagcattga 60
ccggtttaga tcgtaatat cttacaagt taag 94

<210> 25

<211> 94

<212> DNA

<213> Artificial Sequence

<220>

<223> sequence of the dsRNA encoding region of 42 bp for silencing expression of the PDS gene (PDS42)-lower strand

<400> 25
 tcgacttaac ttgtaaggaa tattacgata ctaaccgggc aatgctcctc ctagcattga 60
 ccggttagga tcgtaatat ccttacaagt taag 94

<210> 26

<211> 52

<212> DNA

<213> Artificial Sequence

<220>

<223> sequence of the dsRNA encoding region of 21 bp for silencing expression of the PDS gene (PDS21)-upper strand

<400> 26
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<210> 27

<211> 52

<212> DNA

<213> Artificial Sequence

<220>

<223> sequence of the dsRNA encoding region of 21 bp for silencing expression of the PDS gene (PDS21)-lower strand

<400> 27
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<210> 28

<211> 115

<212> DNA

<213> Artificial sequence

<220>

<223> small hairpin RNA coding region (GUS_A)

<220>

<221> misc_feature

<222> (1)..(11)

<223> SalI/PvuI restriction sites

<220>

<221> misc_feature

<222> (12)..(53)

<223> sense RNA encoding region

<220>

<221> misc_feature

<222> (54)..(62)

<223> loop structure

<220>

<221> misc_feature

<222> (63)..(104)

<223> antisense RNA encoding region

<220>

<221> misc_feature

<222> (105)..(115)

<223> SalI/PvuI restriction sites

<400> 28

gtcgacgac gtgcggtcac tcattacggc aaagtgtggg tcaataatca ggagttcctt 60
cttcctgatt attgacccac actttgccgt aatgagtgcg cgcagtcgac gatcg 115

<210> 29

<211> 112

<212> DNA

<213> Artificial sequence

<220>

<223> small hairpin RNA coding region (GUS_B)

<220>

<221> misc_feature

<222> (1)..(8)

<223> SalI/PvuI restriction sites

<220>

<221> misc_feature

<222> (9)..(50)

<223> sense RNA encoding sequence

<220>

<221> misc_feature

<222> (51)..(59)

<223> loop structure

<220>

<221> misc_feature

<222> (60)..(101)

<223> antisense RNA coding region

<220>

<221> misc_feature

<222> (102)..(112)

<223> SalI/pvuI restriction site

<400> 29

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gtcgacgatc gtcataaaga tgcggacttg cgtggcaaag gattcgataa gttccttctt    60
tatcgaatcc ttgccaacgc aagtcgcgat cttcatgacg agtcgacgat cg          112

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<210> 30

<211> 115

<212> DNA

<213> Artificial sequence

<220>

<223> small hairpin RNA coding region (GUS_C)

<220>

<221> misc_feature

<222> (1)..(11)

<223> SalI/PvuI restriction sites

<220>

<221> misc_feature

<222> (12)..(53)

<223> sense RNA coding region

<220>

<221> misc_feature

<222> (54)..(62)

<223> loop structure

<220>

<221> misc_feature

<222> (63)..(104)

<223> antisense RNA encoding region

<220>

<221> misc_feature

<222> (105)..(115)

<223> SalI/PvuI restriction sites

<400> 30

gtcgcacgac gtgcgacctc gcaaggcata ttgcgcgcttg gcggtaacaa gaagttcctt 60
ctttcttggt accgccaacg cgcaatatgc cttgcgaggt cgcagtcgac gatcg 115

<210> 31

<211> 115

<212> DNA

<213> Artificial sequence

<220>

<223> small hairpin RNA coding region (EIN_A)

<220>

<221> misc_feature

<222> (1)..(11)

<223> SalI/PvuI restriction sites

<220>

<221> misc_feature

<222> (12)..(53)

<223> sense RNA encoding region

<220>

<221> misc_feature

<222> (54)..(62)

<223> loop structure

<220>

<221> misc_feature

<222> (63)..(104)

<223> antisense RNA encoding region

<220>

<221> misc_feature

<222> (105)..(115)

<223> SalI/PvuI restriction sites

<400> 31

gtcgacgacg gcatcttatg ccaatatggt gcagctcgca taagcggtgt gacgttcctt 60
ctgtcacaac gcttatgcga gctgcaacat attggcataa gatggtcgac gatcg 115

<210> 32

<211> 112

<212> DNA

<213> Artificial sequence

<220>

<223> small hairpin RNA coding region (EIN_B)

<220>

<221> misc_feature

<222> (1)..(8)

<223> SalI/PvuI restriction sites

<220>

<221> misc_feature

<222> (9)..(50)

<223> sense RNA coding region

<220>

<221> misc_feature

<222> (51)..(59)

<223> loop structure

<220>

<221> misc_feature

<222> (60)..(101)

<223> antisense RNA coding region

<220>

<221> misc_feature

<222> (102)..(112)

<223> SalI/PvuI restriction site

<400> 32

gtcgacgac	g	g	c	a	g	g	c	c	t	g		g	t	a	t	a	c	t	t	c		t	c	t	a	t	g	t	t	c		t	g	g	c	g	t	c	t	t	g		g	t	t	c	c	t	t	c	t	c		60
a	a	a	c	a	t	a	g	a	g		a	a	g	t	a	a	t	a	c		a	g	g	c	c	t	g	c	c	g		a	g	t	c	g	a	c	g	a	t		c	g		112								

<210> 33

<211> 115

<212> DNA

<213> Artificial sequence

<220>

<223> small hairpin RNA coding region (EIN_C)

<220>

<221> misc_feature

<222> (1)..(11)

<223> SalI/PvuI restriction site

<220>

<221> misc_feature

<222> (12)..(53)

<223> sense RNA encoding region

<220>

<221> misc_feature

<222> (54)..(62)

<223> loop structure

<220>

<221> misc_feature

<222> (63)..(104)

<223> antisense RNA encoding region

<220>

<221> misc_feature

<222> (105)..(115)

<223> SalI/PvuI restriction site

<400> 33

gtcgacgac	gcatagctgt	ttcctgtgtg	aaattggtat	ccgctcacia	ttcgttcctt	60
ctgaattgtg	agcggatacc	aatttcacac	aggaaacagc	tatggtcgac	gatcg	115